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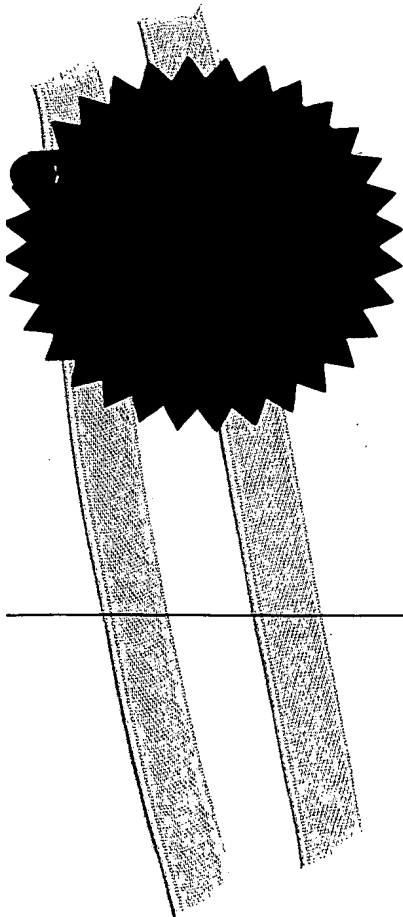
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**בקשה לפטנט**  
Application For Patent

מספר: Number	126452
תאריך: Date	04-10-1998
הוקדם/נדחה: Ante/Post-dated	

אני, (שם המבקש, מענו ולגבי גוף מאוגדת מקום התאגדותו)  
I. (Name and address of applicant, and in case of body corporate-place of incorporation)

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ששמה הוא Inventor

of an invention the title of which is

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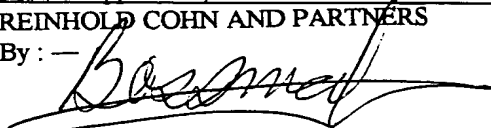
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(English)

A novel lymphocyte population

Hereby apply for a patent to be granted to me in respect thereof.

מבקש בזאת כי ינתן לי עליה פטנט

<b>• בקשת חלוקה •</b> Application of Division <b>מבקשת פטנט</b> from application No. _____ מס' _____ Dated _____ מיום _____		<b>• בקשת פטנט מוסף •</b> Appl. for Patent of Addition <b>מבקשה/לפטנט</b> to Patent/Appl. No. _____ מס' _____ dated _____ מיום _____		<b>דרישת דין קדימה •</b> Priority Claim <table border="1"> <tr> <td>מספר/סימן Number/Mark</td> <td>תאריך Date</td> <td>מדינת האיגוד Convention Country</td> </tr> <tr> <td></td> <td></td> <td></td> </tr> </table>			מספר/סימן Number/Mark	תאריך Date	מדינת האיגוד Convention Country			
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P.O.A.: <b>Special</b> To be filed		<b>• יפוי כח : מיוחד •</b> עוד יוגש										
המען למסירת מסמכים בישראל Address for Service in Israel  REINHOLD COHN AND PARTNERS Patent Attorneys P.O.B. 4060, Tel-Aviv		<b>C. 111889</b>										
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אוכלוסית לימפוציטים חדשה

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**A novel lymphocyte population**

Boris Tartakovsky

בוריס טרטקובסקי

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**C.111889**

## A NOVEL LYMPHOCYTE POPULATION

### FIELD OF THE INVENTION

The invention concerns a novel population of lymphocytes and further concerns use of said lymphocytes for the diagnosis of infections.

### 5 PRIOR ART

The following is a list of prior art publications referred to in the present specification.

1. Simmons, David, L., *et al.*, *Blood*, 73:284-289, 1988.
- 10 2. Goyert, S.M., *et al.*, *Science*, 239:497-400, 1988.
3. Haziot A., *et al.*, *J. Immunol.*, 141:547-552, 1988.
- 15 4. Lauener, R.P., WO Patent Application No. 93632418.
5. Hans, *et al.*, WO Patent Application No. 9525117.
6. Dietzh, *et al.*, German Patent Application No. DE 4237502.
- 20 7. Landsorf, P. and Thomas, T., Canadian Patent Application No. 2191655.
8. Calder, V.L. *et al.*, *5<sup>th</sup> International Conference on Human Leukocyte Differentiation Antigens, Boston, Mass., U.S.A., 1993*
- 25 9. Bernard, A., *et al.*, *Leukocyte Typing: Berlin*, 108:425-426, 428, 1984.

10. McMichael A.J., *Leukocyte Typing III, White Cell Differentiation Antigens*, 586-589, 1987.
11. Todd, R.F., *et al.*, *Hybridoma*, 1:329-337, 1982.
- 5 12. Todd, *et al.*, *J. Immunol.*, 126:1435-1442, 1981.
13. Todd, R.F., and Schlossman, S.F., *Blood*, 59:775-786, 1982.
- 10 14. Todd, *et al.*, *Leukemia Res.*, 5:491-495, 1981.

The acknowledgement herein of the above art should not be construed as an indication that this art is in any way relevant to the patentability of the  
15 invention as defined in the appended claims.

The above publications will be acknowledged in the following by indicating their number from the above list.

## BACKGROUND OF THE INVENTION

Identification of specific cell types and populations is necessary and  
20 desired for correct diagnosis of various disorders and diseases and enables more correct and efficient prognosis and treatment of such conditions.

Many specific populations and sub-populations of lymphocytes are known today which are identified typically by use of monoclonal antibodies that are capable of specifically recognizing antigens expressed by such cells.

25 CD14 is a glycosyl-phosphatidylinositol-linked single-chain surface membrane glycoprotein with a molecular weight of about 55 kDa<sup>(1)</sup>. It is expressed to a high extent on monocytes, macrophages and to a low extent on neutrophils. It has been shown to be very weakly expressed on the outer membrane of certain B-cell lymphocytes, but is absent from T-cells, null cells,  
30 ~~red blood cells and platelets. CD14 was also described to be expressed on the~~  
membrane of Langerhans cells, follicular dendritic cells and histiocytes, and to be present in a soluble form in serum. CD14 has been shown to be a receptor

for lipopolysachharide (LPS) and is considered to be a myelomonocytic differentiation antigen<sup>(2,3)</sup>.

Anti CD14 MABs have been shown to induce IL-10 secretion from CD14 expressing T-cells or by inducing T-cell tolerance or energy<sup>(4)</sup>.  
5 Anti-CD14 antibodies were also described for use in the prevention or treatment of various bacterial infections such as toxemia associated with gram-positive bacteria infection, especially sepsis<sup>(5)</sup>. Medicaments containing anti-idiotypic CD14 antibodies having high endotoxin binding capacity were described for the treatment of infection and other disorders<sup>(6)</sup>.

10 Compositions comprising a combination of a number of antibodies including anti-CD14 antibodies have also been described for use in the production of enriched human blood cell preparations as well as for the enrichment of non-hematopoietic metastatic tumor cells<sup>(7)</sup>.

A number of CD14 epitopes have been reported, each being recognized  
15 by a specific MAb. Such Mabs are, for example, My4<sup>(8, 10)</sup>, CMRF-31<sup>(8)</sup> and MO2<sup>(9-14)</sup>. Intracytoplasmic CD14 protein recognized by the My4 and CMRF-31 antibodies was reported in some non myeloid cells<sup>(8)</sup>.

The MO2 MAb has been shown to react with 58%-84% of peripheral blood monocytes. The MO2 antigen was shown to be expressed on 6%-16% of  
20 bone marrow cells, on mature monocytes and on certain macrophages, but is not known to be expressed by T or B lymphocytes or by granulocytes.

## SUMMARY OF THE INVENTION

In accordance with the present invention, a novel population of lymphocytes was surprisingly discovered comprising cells that express  
25 intracellularly, an antigen recognized by the MO2 Mab. Such cells will be referred to hereinafter as "*MO2<sup>+</sup> cells*", and the antigen expressed intracellularly by such cells will be referred to as "*MO2 antigen*".

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The novel population of cells was detected using the known MO2 antibody and the expression of this antigen in these cells was detected following their fixation and permeabilization. No expression of the MO2 antigen was detected on the cell surface of these cells. Therefore, unless  
5 specifically stated otherwise, reference to the expression of the MO2 antigen by cells will refer to intracellular expression of the antigen.

The novel cell population of the invention was found to comprise two subpopulations. One subpopulation of cells, in addition to expressing the anti MO2 antigen internally, was also found to express the general T-cell antigen  
10 marker CD3 and therefore this population of cells is considered to be a T-cell population of lymphocytes and will be referred to hereinafter as CD3<sup>+</sup> MO2<sup>+</sup> cells. In accordance with the invention, it was also found that a large percent of T-cells in peripheral blood which express the CD8 antigen (characterizing mainly suppressor and cytotoxic T-cells) also express the MO2 antigen  
15 intracellularly. Only a small percent of peripheral blood T-cells expressing the CD4 antigen (characterizing T-helper cells) were found to express the MO2 antigen intracellularly.

T-cells expressing the gamma/delta T-cell receptor (TCR) are believed to be involved in the etiology of infectious diseases. In accordance with the  
20 invention, it has been shown that most of these T-cells expressing the gamma/delta TCR also express the MO2 antigen intracellularly.

The second novel subpopulation of lymphocytes which, in accordance with the invention were found to express the MO2 antigen internally, are characterized as non-T, non-B cells. At least some of these cells express the  
25 CD16 antigen marker (and thus may be NK-like cells).

The present invention thus provides a novel population of lymphocytes which express intracellularly a protein or polypeptide capable of binding to an antibody being a member of the group consisting of:

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- (i) anti MO2 MAbs;

(ii) fragments of the antibodies of (i) above which essentially retain the antigen binding characteristics of the non fragmented anti MO2 MAb;

(iii) antibodies capable of binding to the antigenic epitope bound by any one of the antibodies of (i) or (ii) above;

5 said lymphocytes having no expression of the MO2 antigen on their surface.

By a preferred embodiment, the novel population of MO2<sup>+</sup> cells in accordance with the invention are T-cells expressing the CD3 antigen on their cell surface and expressing the MO2 antigen intracellularly, hereinafter CD3<sup>+</sup> MO2<sup>+</sup>. The expression of the CD3 antigen on these cells can be detected by  
10 any of the methods known in the art using any of the anti-CD3 MoAbs available such as, for example, the MoAb manufactured by Coulter Immunnology.

The present invention further provides a novel population of non-T  
15 non-B MO2<sup>+</sup> lymphocytes. Such cells may be detected by their positive reaction after fixation and permeabilization with the anti MO2 MAb, as well as by their positive reaction with anti CD16 MoAb. These cells are typically negative in their reaction with anti-CD3 MAbs as well as their negative reaction with anti-CD22 MAbs.

20 Moreover, in accordance with the invention, a significant increase was shown in the percent of MO2 T-cells and or in the level of MO2 expression in peripheral blood cells of individuals suffering from infections of viral or bacterial origin as compared to the percent of such cells and/or level of intracellular MO2 in the same cells in peripheral blood of healthy individuals.  
25 Further analysis showed that the most significant increase was in the MO2<sup>+</sup> CD3<sup>+</sup> cells of the infected individuals.

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The present invention further provides a method for detection of an infection in a tested individual comprising the steps of:



- (i) obtaining a peripheral blood sample from said individual to be tested;
- (ii) separation of mononuclear cells from said peripheral blood;
- (iii) fixation and permeabilization of said mononuclear cells;
- 5 (iv) incubation of said fixed and permeabilized mononuclear cells with MAbs capable of binding to the MO2 antigen under conditions enabling binding of the Mabs to said antigen;
- 10 (v) detecting binding of said antibodies in said cells, and determining the number of cells in said sample expressing the MO2 antigen internally and/or the intracellular level of MO2 expression in said cells; a higher expression of MO2 in said cells as compared to MO2 expression in a control non-infected individual, indicating the existence of an infection in said tested individual.

In accordance with a preferred embodiment of the above aspects of the  
15 invention, following with or simultaneously with the incubation of the fixed and permeabilized mononuclear cells with anti MO2 MAbs, the cells are also incubated with MAbs capable of binding to the CD3<sup>+</sup> antigen under conditions enabling binding of these MAbs to said antigen. Following double staining of the cells, the number of CD3<sup>+</sup> MO2<sup>+</sup> cells in said sample as well as the level of  
20 the MO2 expression in the CD3<sup>+</sup> cells are determined; a higher number of CD3<sup>+</sup> MO2<sup>+</sup> cells and/or a higher level of MO2 expression in said CD3<sup>+</sup> cells compared to the number of MO2<sup>+</sup> CD3<sup>+</sup> cells and/or the level of MO2 in CD3<sup>+</sup> cells in a control non-infected individual, indicating the existence of an infection in said tested individual.

25 The term "*higher expression*" denotes a higher number of cells expressing the MO2 antigen intracellularly and/or a higher level of intracellular MO2 detected in the tested cells.

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In accordance with the invention, the term "*infection*" relates to any infection of a bacterial origin such as, for example, pneumonia or urinary track

infection (UTI), which often results in sepsis. In addition, an infection may be of a viral origin such as, for example, HIV infection.

The separation of mononuclear cells from peripheral blood obtained from a tested individual may be carried out by any of the methods known in the art such as for example separation on a Ficoll-Hypaque gradient.

The detection of the MO2 antigen in accordance with the invention may be obtained by using any antibody capable of binding to the MO2 antigen such as, for example, the MO2-RD1 antibody used in the examples below. The antibody used may be directly labeled with a detectable marker such as radioactive or fluorescent marker. Alternatively, the anti MO2 antibody may be detected by using a secondary antibody comprising a detectable label.

In accordance with the invention, the anti MO2 antibody used for detecting the MO2 antigen may be a whole antibody or alternatively, fragments of the anti MO2 Mab having similar binding properties. Such Mab fragments may be for example, an Fab fragment or an  $F(ab')_2$  fragment obtained by cleavage with appropriate enzymes as known in the art. Fragments which may be useful in accordance with the invention may be screened by competitive binding assays with the respective whole anti MO2 MAb and those which are capable of competing with such binding, i.e. bind to the MO2 antigen may be used. The MAbs in accordance with the invention may belong to any of the various immunoglobulin classes.

The binding of the anti MO2 MAb may be detected in accordance with the invention by any of the methods known in the art such as FACS, RIA, ELISA, immunoblotting, etc. For detection of intracellular expression of the MO2 antigen in the cells in accordance with the invention, the tested cells are fixed and permeabilized prior to their staining with the relevant MAb by methods known in the art.

The detection of the CD3 antigen in the tested cells may be carried out by using any of the anti-CD3 antibodies known in the art as well as any

fragments thereof or labeled anti-CD3 antibodies who essentially maintain the binding characteristics of the whole non labeled anti-CD3 antibody. The detection of the binding of such antibodies to the cells may be carried out by any of the methods known in the art such as those mentioned above.

5 In addition, the cells which were found in accordance with the invention to express the MO2 antigen intracellularly are, typically, in an activated state. The internal expression of the MO2 antigen in the cells may be a result of an internal signal in the cells upon their activation or may result from the cells' response to an external signal of some kind (e.g. soluble MO2 antigen) and this  
10 signal may, in some cases, be taken up by the cells from their environment.

In accordance with the invention, it has been shown that the intracellular expression of the MO2 antigen in MO2<sup>+</sup> cells may be detected even in relatively slightly activated cells, e.g. in cells following their growth in culture (see example below). Thus, the intracellular expression of the MO2 antigen  
15 may be useful as a sensitive indication of activation, e.g. following injection of an antigen to an individual for detection of the development of a primary immune response in the injected individual.

The present invention thus provides by a further of its aspects a method for the detection of activated lymphocytes in a tested individual  
20 comprising the steps of:

- (i) obtaining a peripheral blood sample from said individual to be tested;
  - (ii) separation of mononuclear cells from said peripheral blood;
  - (iii) fixation and permeabilization of said mononuclear cells;
  - 25 (iv) incubation of said fixed and permeabilized mononuclear cells with MAbs capable of binding to the MO2 antigen under conditions enabling binding of the MAbs to said antigen;
- 
- (v) following incubation of said cells in (iv) above or simultaneously therewith, incubating said fixed and permeabilized mononuclear

cells with MAbs capable of binding to the CD3 antigen under conditions enabling binding of the MAbs to said antigen; and

- (vi) detecting binding of said antibodies in said cells, and determining the number of CD3<sup>+</sup> cells in said sample expressing the MO2 antigen intracellularly and/or the level of intracellular expression of MO2 in said CD3 cells; a higher number of CD3<sup>+</sup> MO2<sup>+</sup> cells or a higher level of intracellular MO2 expression in said CD3<sup>+</sup> cells as compared to the number of said cells or the level of expression of MO2 in said cells higher than that of a control non-effective individual, indicating the existence of activated lymphocytes in said tested individual.

In accordance with one embodiment of this aspect of the invention, the above method will be carried out in an individual following immunization, in order to detect and monitor the development of an immune reaction in the immunized individual. The invention thus provides a method for determining the development of an immune response in an immunized individual, comprising:

- (i) injecting an antigen to the tested individual;
- (ii) obtaining a peripheral blood sample from said individual to be tested;
- (iii) separation of mononuclear cells from said peripheral blood;
- (iv) fixation and permeabilization of said mononuclear cells;
- (v) following incubation of said cells in (iv) above or simultaneously therewith, incubating said fixed and permeabilized mononuclear cells with MAbs capable of binding to the CD3 antigen under conditions enabling binding of the MAbs to said antigen; and
- (vi) ~~detecting binding of said antibodies in said cells, and determining~~ the number of CD3<sup>+</sup> cells in said sample expressing the MO2 antigen intracellularly and/or the level of intracellular expression

of MO2 in said CD3 cells; a higher number of CD3<sup>+</sup> MO2<sup>+</sup> cells or a higher level of intracellular MO2 expression in said CD3<sup>+</sup> cells as compared to the number of said cells or the level of expression of MO2 in said cells higher than that of a control non-effective individual, indicating a high probability of the development of an immune response in said tested individual.

In addition, the present invention provides a kit comprising antibodies capable of binding to the MO2 antigen together with reagents necessary for fixation, and permeabilization of the tested cells and, optionally, antibodies capable of binding to the CD3 antigen and means for detecting said binding of said MAbs to the tested cells.

#### EXAMPLES

The invention will now be illustrated further by the following examples with occasional reference to the figures. The examples are intended for illustration purposes only and are not to be construed as limiting.

#### BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the results of fluorescence analysis by FACS of mononuclear cells obtained as described below which were first fixed and permeabilized as explained below, and then stained using one or more of the following MAbs:

Fig. 1A MY4-FITC

Fig. 1B MO2-RD1

Fig. 1C IgG2-FITC (control for MY4-FITC); and

Fig. 1D IgM-RD1 (control for the MO2-RD1 MAb).

The figure shows the level of fluorescence on the stained cells as well as the size of the cell. The cells are considered to be positively stained wherein the

level of fluorescence of the cells is above the fluorescence level of the same cells after their incubation with the control MABs.

**Fig. 2** shows FACS analysis of cells stained by the MABs mentioned in Fig. 1 without fixation or permeabilization of the cells prior to staining, i.e. the  
5 membrane expression of the antigens.

**Fig. 3** shows results of FACS analysis of lymphocytes stained with the control IgM-RD1 Mab or MO2-RD1 MAb either after fixation only (Figs. 3A and 3C) or after fixation and permeabilization (Figs. 3B and 3D).

**Fig. 4A** shows results of FACS analysis of lymphocytes following  
10 double staining of the cells with the MO2-RD1 MAb and anti CD3-FITC MAb;

**Fig. 4B** shows FACS analysis of the lymphocytes stained with the control Mab IgM-RD1 and the anti CD3-FITC MAb.

**Fig. 5** shows the results of FACS analysis of double staining of  
15 lymphocytes with the MO2-RD1 MAb and anti CD8-FITC MAb (Fig. 5A). IgM-RD1 was used as control MAb (Fig. 5B).

**Fig. 6** shows results of FACS analysis of double staining of the lymphocytes with the MO2-RD1 MAb and an anti CD4-FITC MAb (Fig. 6A) the IgM-RD1 antibody was used as control MAb (Fig. 6B).

**Fig. 7** shows FACS analysis of double staining of lymphocytes with the  
20 MO2-RD1 MAb and anti gamma/delta TCR-FITC MAb (Fig. 7A). The IgM-RD1 antibody was used as control (Fig. 7B).

**Fig. 8** shows FACS analysis of double staining of lymphocytes with the MO2-RD1 MAb and an anti CD22-FITC MAb (Fig. 8A). The IgM-RD1 MAb  
25 was used as a control Mab (Fig. 8B).

**Fig. 9** shows the results of FACS analysis of lymphocytes stained with  
~~the anti CD16 FITC MAb and MO2-RD1 MAb (Fig. 9A). The IgM-RD1 MAb~~  
was used as a control MAb (Fig. 9B).

**Fig. 10** is a schematic representation showing the percent of the lymphocytes expressing the MO2 antigen intracellularly in mononuclear blood cells obtained from individuals suffering from various kinds of infections (black bars in the fig.) as compared to the presence of MO2<sup>+</sup> cells obtained from healthy control individuals (grey bars in the fig.). The expression of the MO2 antigen was detected using the MO2-RD1 MAb on fixed and permeabilized cells as described below. The results show the total MO2<sup>+</sup> lymphocyte population, the MO2<sup>+</sup>CD3<sup>+</sup> lymphocyte population and the MO2<sup>+</sup> CD3<sup>-</sup> lymphocyte population in both above groups of individuals.

**Fig. 11** is a schematic representation showing the percent of MO2<sup>+</sup> CD3<sup>+</sup> cells in ten individuals suffering from various kinds of infections as compared to the percent of these cells in healthy individuals.

**Fig. 12** is a schematic representation showing the percent (and mean percent + S.D.) of MO2<sup>+</sup> CD8<sup>+</sup> cells in four individuals suffering from various kinds of infections as compared to the percent (and mean percent + S.D.) of MO2<sup>+</sup> CD4<sup>+</sup> cells in the same individuals.

## MATERIALS AND METHODS

### Fixation and permeabilization of lymphocytes:

Peripheral blood is obtained from individuals to be tested and mononuclear cells are separated on a ficoll-hypaque gradient. The cells are then washed in phosphate buffered cell line (PBS) and then fixed in 4% paraformaldehyde in PBS for 30 mins. at 4°C. The cells are then washed again in PBS and permeabilized in PBS supplemented with 0.1% saponin and 1% bovine serum albumin for 30 mins. at room temperature.

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### Staining:

The cells prepared as described above, are then divided into staining tubes and processed in the saponin buffer for 1 or 2-color fluorescent staining.

The cells are incubated with the tested monoclonal antibody or with the control monoclonal antibody for 30 mins. at room temperature. Staining can be done either by direct staining, i.e. by incubating the cells with a labeled Ab, or indirectly, i.e. by incubating the cells with first non-labeled Ab and then with a secondary labeled Ab. Following incubation, the cells are washed once in saponin buffer and twice in PBS. The level of staining is then determined using the FACS as described below.

#### Monoclonal antibodies:

10 The MAbs used to determine the expression of various antigens in the tested cells are the following:

The anti-CD14 MAbs anti-MY4 and anti-MO2 as well as the Anti-CD3, Anti-CD4, Anti-CD8, and Anti-CD16 were all obtained from Coulter Immunology. The anti-TCR, alpha beta, and anti-TCR gamma delta MAbs  
15 were obtained from Immunotech.

#### Fluorescence-activated cell Sorter-FACS

The analysis of the intracellular or membrane expression of the MO2 antigen is performed with the use of the FACS (model FACSCAN  
20 manufactured by Becton Dickinson).

Cells of the lymphocyte or monocyte lineage can be detailed and characterized according to their light-scattering properties. Their scatter positions, determined by their different forward and side laser- light scatters, enable to analyze both populations independently, by applying a "gate" or  
25 window on the cells of interest. In addition, the instrument is capable of measuring the fluorescence of the cells, either the green fluorescence, emitted by FITC, or the red fluorescence, emitted by RD1 - two different dyes conjugated to the MAbs. On the basis of the fluorescence of the cells, data can be obtained on the number of fluorescent cells, above background, in the



population of cells under analysis, and also on the level of intensity of the fluorescence, a measurement directly correlated with the amount per cell of the antigen detected by the conjugated MAb.

5 **RESULTS:**

**Example 1: Intracellular expression of MO2 in lymphocytes**

As seen in Fig. 1, the novel population of lymphocytes which express the MO2 antigen internally was detected using the MO2-RD1 antibody on fixed and permeabilized mononuclear cells which were obtained as described  
10 above. No expression of an antigen recognized by the MY4-FITC antibody was detected on the same cells. As seen in Fig. 2, no expression of the MO2 antigen or the MY4 antigen was detected in the same cells which were not fixed and permeabilized, i.e. the MO2 antigen which was expressed internally on the cells was not detected on their surface.

15 As seen in Figs. 1 and 2, the MO2 antigen was expressed to some extent on the cell surface of monocytes.

**Example 2: Detection of intracellular expression of MO2 on lymphocytes by fixation and permeabilization.**

20 Mononuclear cells obtained as explained above, were divided into the following two groups:

1. Cells that underwent fixation only as described above;
2. Cells that were fixed and also permeabilized as described above.

Both groups of cells were incubated with the MO2-RD1 MAb and the  
25 expression of MO2 was analyzed by FACS.

As seen in Fig. 3, the MO2 antigen was detected only in the above group 2 lymphocytes and no antigen was detected in cells of group 1. The  
above results show that the cells express the MO2 antigen internally only and

that detection of the MO2 antigen in these cells by the Anti-MO2 MAbs requires permeabilization of the cells.

**Example 3: The MO2<sup>+</sup> cells comprise two subpopulations:**

- 5           3.1    Mononuclear cells obtained as explained above were double stained with an anti-MO2-RD1 antibody and an anti-CD3-FITC MAb and the level of fluorescence was measured by FACS as explained above. As seen in Fig. 4, the MO2<sup>+</sup> cells comprise two subpopulations: those which do not express the CD3 antigen (non T cells, hereinafter CD3<sup>-</sup> MO2<sup>+</sup> (Fig. 4A) and those cells
- 10                   expressing the CD3 antigen (T lymphocytes, hereinafter CD3<sup>+</sup> MO2<sup>+</sup> (Fig. 4B).
- 3.2    Characterization of the CD3<sup>+</sup> MO2<sup>+</sup> T lymphocytes:
- 3.2.1 Mononuclear cells were double stained with the anti
- 15                   MO2 MAb and an anti-CD8 MAb (a marker of cytotoxic and suppressor cells). As seen in Fig. 5, a large percent of the CD8<sup>+</sup> cells also express the MO2 antigen internally.
- 3.2.2 The mononuclear cells were double stained with the anti
- 20                   MO2 MAb and an anti-CD4 MAb (a marker for T helper cells). As seen in Fig. 6, only a very small percent of the CD4<sup>+</sup> cells expressed the MO2 antigen internally.
- 3.2.3 The mononuclear cells were double stained with the anti
- 25                   MO2 Mab and with a MAb recognizing the gamma/delta T-cell receptor (about 2% of peripheral blood T cells). As seen in Fig. 7, most of the T cells expressing the gamma/delta type T cell receptor also expressed the MO2 antigen internally.
-

3.3 Characterization of the non T MO2<sup>+</sup> CD3<sup>-</sup> cells:

3.3.1 Mononuclear cells obtained as explained above were double stained with the anti MO2 MAb and an anti-CD22 MAb (which is a marker for mature B lymphocytes). As seen Fig. 8, none of the CD22<sup>+</sup> cells (mature B lymphocytes) were positive for MO2. The above results show that none of the B cells express the MO2 antigen internally. These results also show that the CD3<sup>-</sup> cells which express the MO<sub>2</sub> antigen internally (see 3.1 above) are non T- non B cells.

3.3.2 The cells were double stained with the MO2 Mab and with the anti-CD16 antibody (NK-like cells). As seen in Fig. 9, a large percent of the NK-like cells (CD16<sup>+</sup>) also express the MO2 antigen internally.

**Example 4: Expression of MO2<sup>+</sup> cells in individuals suffering from infection:**

4.1 The percent of the cells expressing the MO2 antigen internally (MO2<sup>+</sup>) in individuals suffering from various infectious diseases of bacterial and viral origin were determined and compared to the percent of the MO2<sup>+</sup> cells in healthy individuals. As seen in Fig. 10, the percent of MO2<sup>+</sup> lymphocytes in individuals suffering from infections was substantially higher than the percent of these cells found number of the cells in healthy individuals. As may be seen, the most substantial rise is in the percent of CD3<sup>+</sup> MO2<sup>+</sup> cells.

4.2 As seen in Fig. 11, the level of MO2<sup>+</sup> CD3<sup>+</sup> cells was found to be higher in individuals suffering from various kinds of infections including bacterial (sepsis) and viral (asymptomatic and

symptomatic HIV+ individuals). The mean percent of MO2<sup>+</sup> CD3<sup>+</sup> cells in healthy individuals was in the range of between about 0% to 15% while the mean percent of these cells in individuals suffering from infection was in the range of between about 20% to about 60%.

5 4.3 In order to characterize the MO2<sup>+</sup> T cells appearing in a higher level in individuals suffering from infections as compared to healthy individuals lymphocytes obtained from four individuals suffering from various infections were also stained with the MO2-RD1 MAb and with anti-CD8 or with anti-CD4 antibodies. 10 As seen in Fig. 12, in each of the four tested individuals, the level of the MO2<sup>+</sup> T cells expressing the CD8 antigen was substantially higher than the percent of MO2<sup>+</sup> T cells expressing the CD4 antigen in the same individual. Therefore, the majority of the MO2<sup>+</sup> T cells which are found at a higher level in 15 individuals suffering from infections seem to be CD8<sup>+</sup> T cells while only the minority of these cells seem to be CD4<sup>+</sup> T cells.

20 **Example 5: Expression of intracellular MO2 in cells activated by growth in culture**

Mononuclear cells, obtained after Ficoll-Hypaque purification, were divided into two groups. Cells in group one were fixed, permeabilized and double stained for intracellular MO2 antigen and for the gamma-delta T-cell receptor antigen, as explained above. Cells in the second group were put in culture (RPMI medium, supplemented 25 with 5% fetal-calf serum, glutamine and antibiotic mix), in a humidified incubator, for 18 hours, at 37 degrees and 5% CO<sub>2</sub>. At the end of the incubation period, cells were washed, fixed, permeabilized and double

stained for intracellular MO2 antigen and for the gamma-delta T-cell receptor antigen, as explained above.

Results, shown in Table 1, represent the net mean intracellular MO2 fluorescence of gamma-delta positive cells, before and after the incubation. The net mean fluorescence channel (obtained from the FACS analysis), correlates with the intracellular amount of the MO2 antigen. As shown, in the majority of the samples analyzed, we observed a significant increase in the amount of the intracellular MO2 antigen in the gamma-delta T-cells, following their stimulation by the 18 hours culture.

**Table 1**

<b><u>Individual #</u></b>	<b><u>Mean Net Fluorescence Channel</u></b> <b>(Correlates with intracellular amount of MO2 antigen)</b>	
	<b>Before Culture</b>	<b>After Culture</b>
1	2.3	4
2	2.5	11.7
3	1.5	12
4	2.7	9.7
5	3	17
6	17.8	20.6
7	0.9	6.6
8	1.5	12.8
9	3	13.3
10	2.7	13.9

CLAIMS:

1. A novel population of lymphocytes which express intracellularly a protein or polypeptide capable of binding to an antibody being a member of the  
5 group consisting of:
  - (i) anti MO2 MAbs;
  - (ii) fragments of the antibodies of (i) above which essentially retain the antigen binding characteristics of the non fragmented anti MO2 MAb;
  - (iii) antibodies capable of binding to the antigenic epitope bound by  
10 any one of the antibodies of (i) or (ii) above;said lymphocytes having no expression of the MO2 antigen on their surface (said cells being designated as MO2<sup>+</sup> cells).
2. A population of lymphocytes in accordance with claim 1, wherein said lymphocytes express the CD3 antigen on their cell surface.
- 15 3. A population of lymphocytes in accordance with claim 1, wherein said lymphocytes express the CD16 antigen on their cell surface.
4. A population of lymphocytes in accordance with claim 3, wherein said lymphocytes have no expression of the CD3 and CD22 antigens on their cell surface.
- 20 5. A method for detection of an infection in a tested individual comprising the steps of:
  - (i) obtaining a peripheral blood sample from said individual to be tested;
  - (ii) separation of mononuclear cells from said peripheral blood;
  - 25 (iii) fixation and permeabilization of said mononuclear cells;
  - (iv) incubation of said fixed and permeabilized mononuclear cells with MAbs capable of binding to the MO2 antigen under conditions permitting binding of said antibody to the said antigen; and

- (v) detecting binding of said antibodies in said cells, and determining the number of cells in said sample expressing the MO2 antigen internally, and/or the level of intracellular MO2 in said cells; a number higher than the number of said cells in a control non-infected individual and/or a level of MO2 antigen higher than that in cells of a non-infected individual, indicating the existence of an infection in said tested individual.

6. A method for detection of an infection in a tested individual comprising the steps of:

- (i) obtaining a peripheral blood sample from said individual to be tested;
- (ii) separation of mononuclear cells from said peripheral blood;
- (iii) fixation and permeabilization of said mononuclear cells;
- (iv) incubation of said fixed and permeabilized mononuclear cells with MAbs capable of binding to the MO2 antigen under conditions permitting binding of said antibody to the said antigen;
- (v) following or simultaneously with incubation of said cells with the anti-MO2 MAbs, incubation of said cells with an antibody capable of binding to the CD3 antigen; and
- (vi) detecting binding of said antibodies in said cells, and determining the number of CD3<sup>+</sup> cells in said sample expressing the MO2 antigen internally and/or the level of intracellular MO2 expression in said CD3<sup>+</sup> cells; a number of said cells or a level of said antigen higher than the number of said cells or the level of said antigen in a control non-infected individual, indicating the existence of an infection in said tested individual.

7. A method in accordance with claims 5 or 6, wherein said infection is a bacterial infection.

8. A method in accordance with claims 5 or 6, wherein said infection is a viral infection.

9. A method in accordance with claim 8, wherein said viral infection is an HIV infection.

5 10. A method for the detection of activated lymphocytes in a tested individual comprising the steps of:

- (i) obtaining a peripheral blood sample from said individual to be tested;
- (ii) separation of mononuclear cells from said peripheral blood;
- 10 (iii) fixation and permeabilization of said mononuclear cells;
- (iv) incubation of said fixed and permeabilized mononuclear cells with MAbs capable of binding to the MO2 antigen under conditions enabling binding of the Mabs to said antigen; and
- 15 (v) detecting binding of said antibodies in said cells, and determining the number of cells in said sample expressing the MO2 antigen internally, and/or the level of intracellular MO2 in said cells; a number higher than the number of said cells in a control non-infected individual and/or a level higher than that in cells of a non-infected individual, indicating the existence of activated
- 20 lymphocytes in said tested individual.

11. A method for the detection of activated lymphocytes in a tested individual comprising the steps of:

- (i) obtaining a peripheral blood sample from said individual to be tested;
- 25 (ii) separation of mononuclear cells from said peripheral blood;
- (iii) fixation and permeabilization of said mononuclear cells;
- (iv) ~~incubation of said fixed and permeabilized mononuclear cells~~ with MAbs capable of binding to the MO2 antigen under conditions enabling binding of the Mabs to said antigen;



(v) following or simultaneously with incubation of said cells with the anti-MO2 MAbs, incubation of said cells with an antibody capable of binding to the CD3 antigen; and

5 (vi) detecting binding of said antibodies in said cells, and determining the number of CD3<sup>+</sup> cells in said sample expressing the MO2 antigen internally and/or the level of intracellular MO2 expression in said CD3<sup>+</sup> cells, a number of said cells or a level of said antigen higher than the number of said cells or the level of said antigens in a control non-infected individual, indicating the  
10 existence of activated lymphocytes in said tested individual.

12. A method for determining the development of an immune response in an immunized individual, comprising:

(i) injecting an antigen to the tested individual;

15 (ii) obtaining a peripheral blood sample from said individual to be tested;

(iii) separation of mononuclear cells from said peripheral blood;

(iv) fixation and permeabilization of said mononuclear cells;

20 (v) incubation of said fixed and permeabilized mononuclear cells with MAbs capable of binding to the MO2 antigen under conditions enabling binding of the Mabs to said antigen; and

(vi) detecting binding of said antibodies in said cells, and determining the number of cells in said sample expressing the MO2 antigen internally and/or the level of intracellular MO2 expression in said cells; a number higher than the number of said cells in a control non-infected individual and/or a level higher than that in cells of  
25 a non-infected individual, indicating a high probability of the development of an immune response in said tested individual.

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13. A method for determining the development of an immune response in an immunized individual, comprising:

- (i) injecting an antigen to the tested individual;
- (ii) obtaining a peripheral blood sample from said individual to be tested;
- (iii) separation of mononuclear cells from said peripheral blood;
- 5 (iv) fixation and permeabilization of said mononuclear cells;
- (v) following or simultaneously with incubation of said cells with the anti-MO2 MAbs, incubation of said cells with an antibody capable of binding to the CD3 antigen; and
- 10 (vi) detecting binding of said antibodies in said cells, and determining the number of CD3<sup>+</sup> cells in said sample expressing the MO2 antigen internally and/or the level of intracellular MO2 expression in said CD3<sup>+</sup> cells, a number of said cells or a level of said antigen higher than the number of said cells or the level of said antigens in a control non-infected individual, indicating a  
15 higher probability of the development of an immune response in said tested individual.

14. A kit comprising antibodies capable of binding to the MO2 antigen and optionally antibodies capable of binding to the CD3 antigen together with reagents necessary for fixation, and permeabilization of the tested cells and  
20 means for detecting binding of said MAb to the tested cells.

15. A kit comprising antibodies capable of binding to the MO2 antigen and antibodies capable of binding to the CD3 antigen together with reagents necessary for fixation, and permeabilization of the tested cells and means for detecting binding of said MAb to the tested cells.

25 16. A population of lymphocytes in accordance with any one of claims 1-4, wherein said anti-MO2 MAbs are MO2-RD1 MAbs, fragments thereof ~~retaining the antigen-binding characteristics of said MAbs or antibodies capable~~ of binding to the antigenic epitope bound by the MO2-RD1 antibodies or fragments thereof.

17. A method in accordance with any of claims 5-13, wherein the MAbs capable of binding to the MO2 antigen are MO2-RDI MAbs.

For the Applicants,

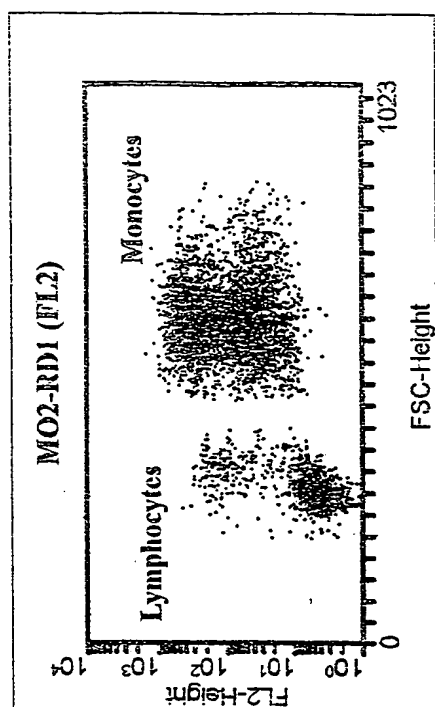
**REINHOLD COHN AND PARTNERS**

By: 

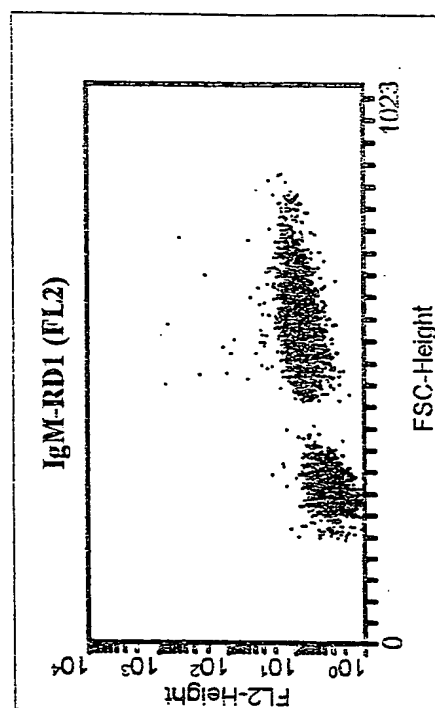
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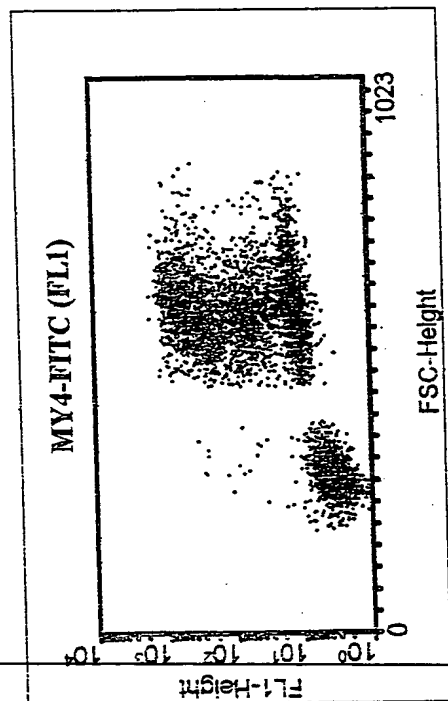
B



D



A



C

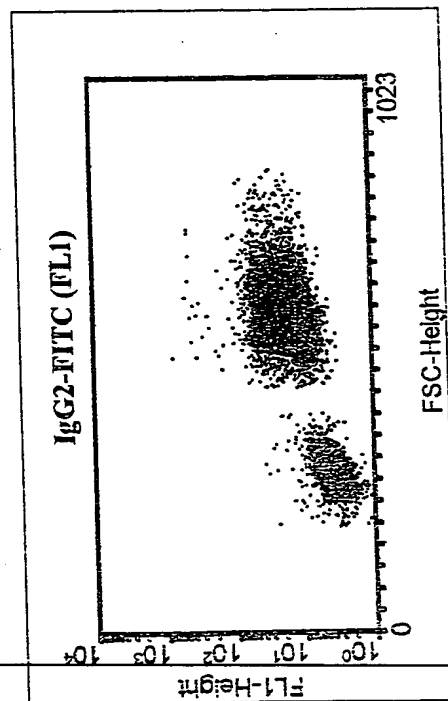
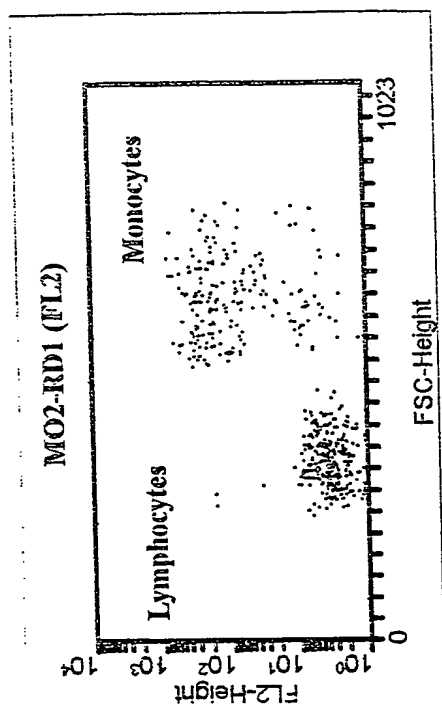
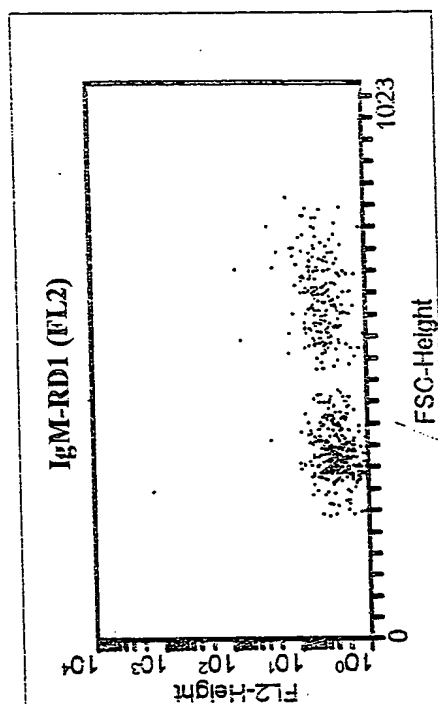


Fig. 1

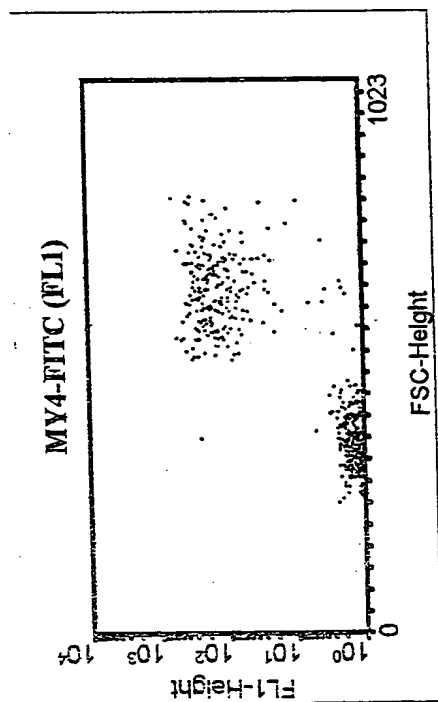
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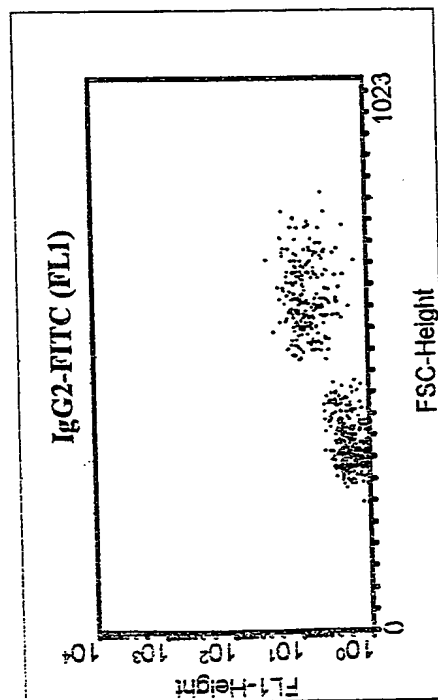
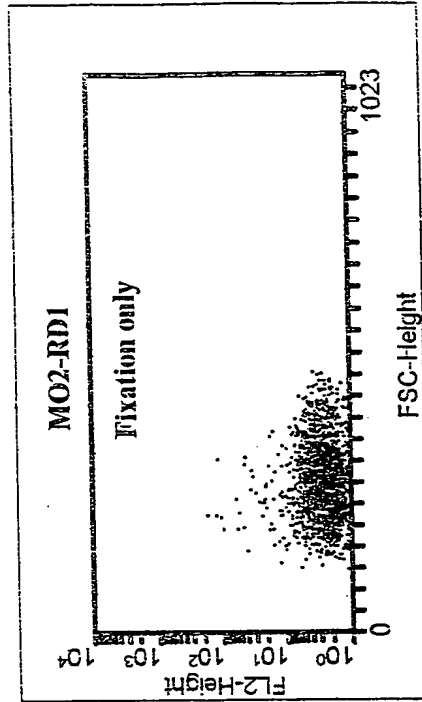
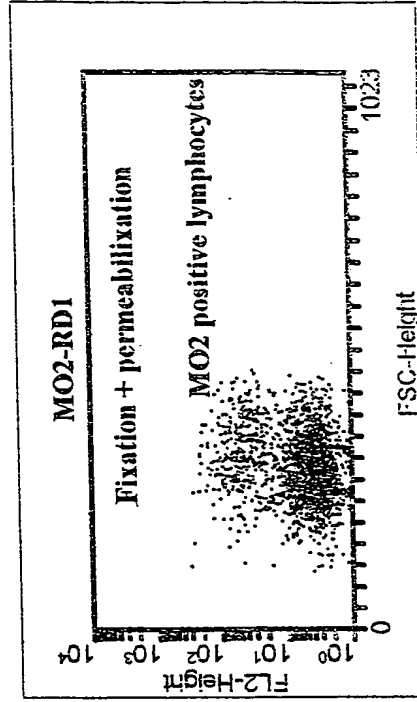


Fig. 2

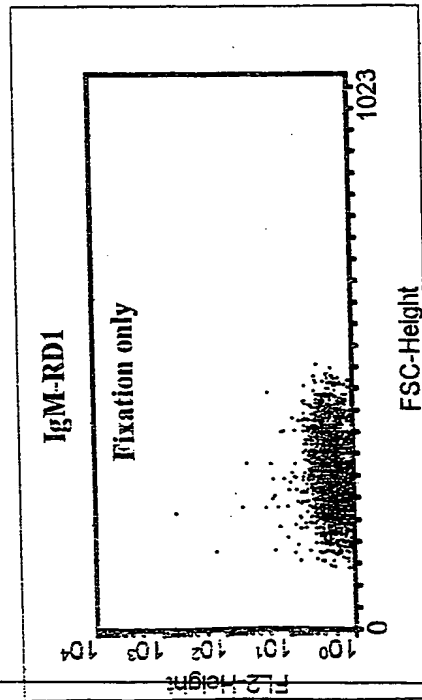
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A



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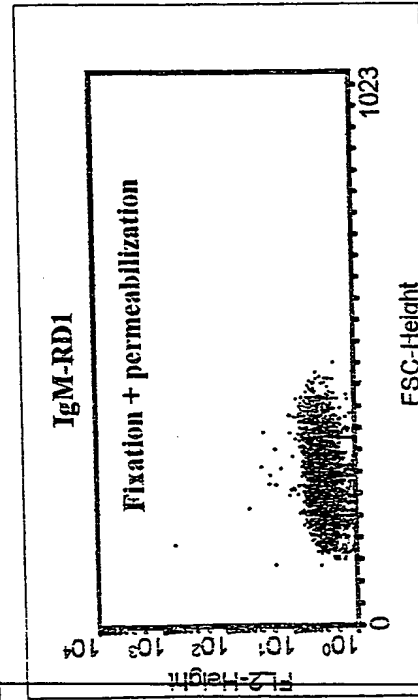


Fig. 3

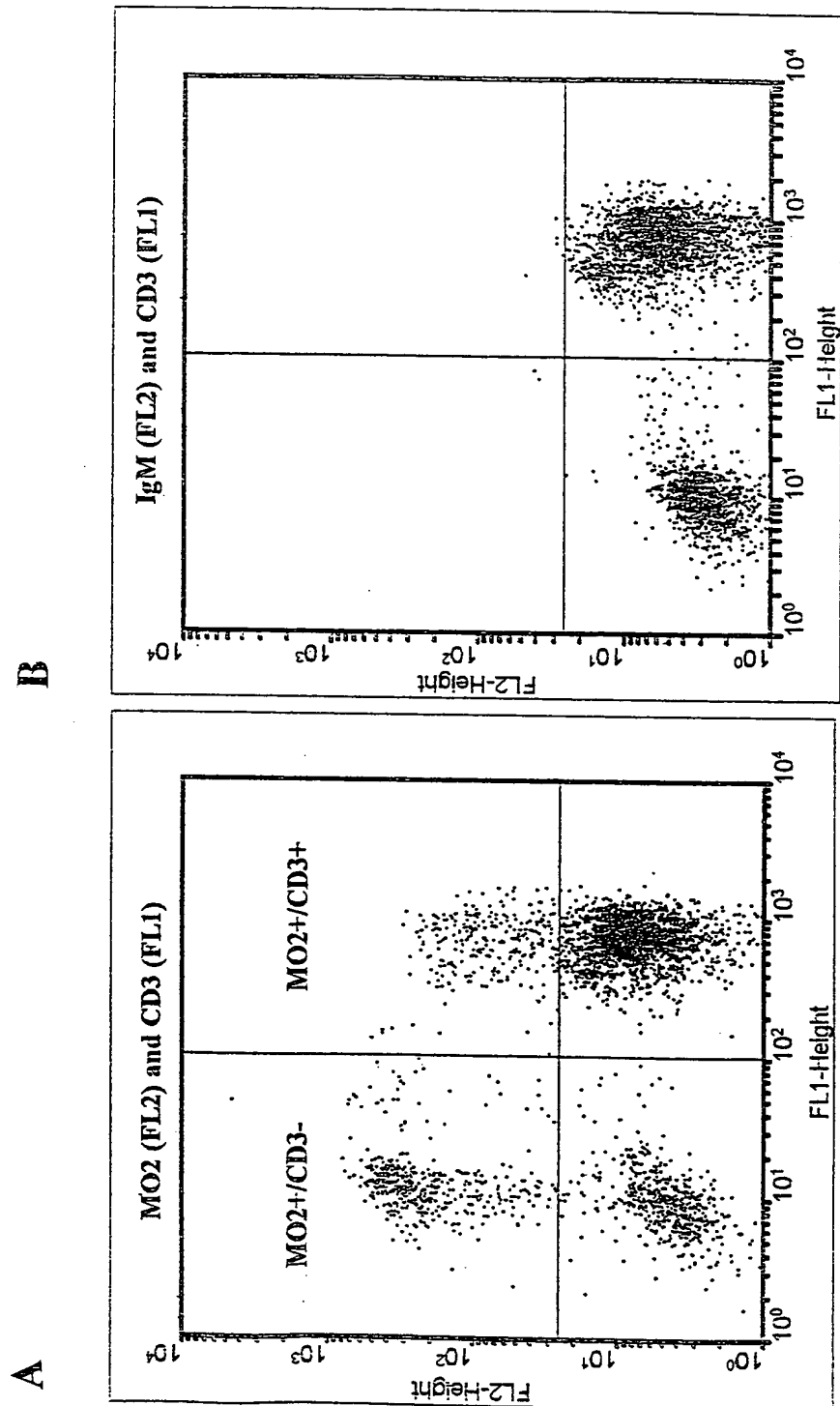


Fig. 4

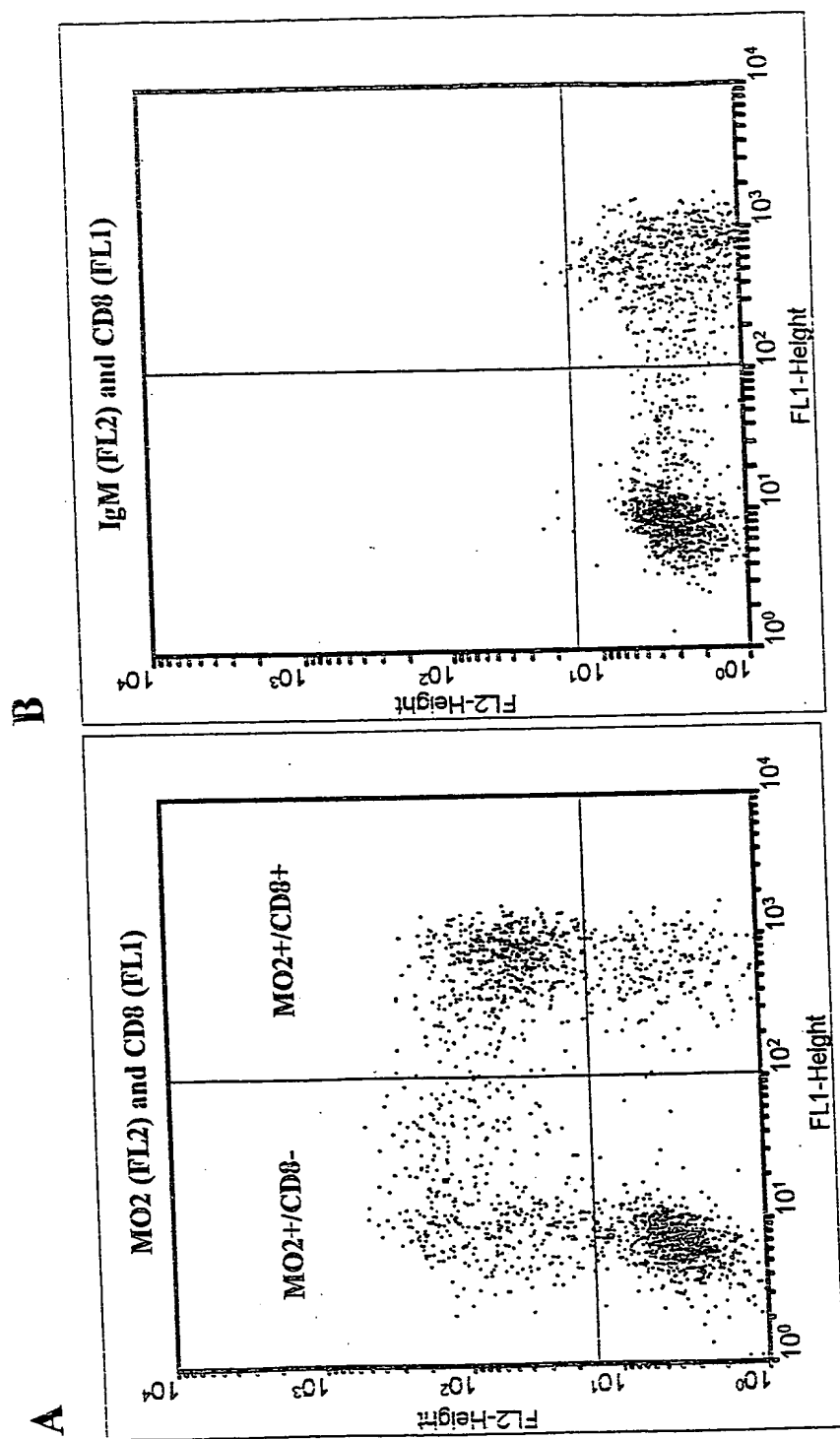


Fig. 5



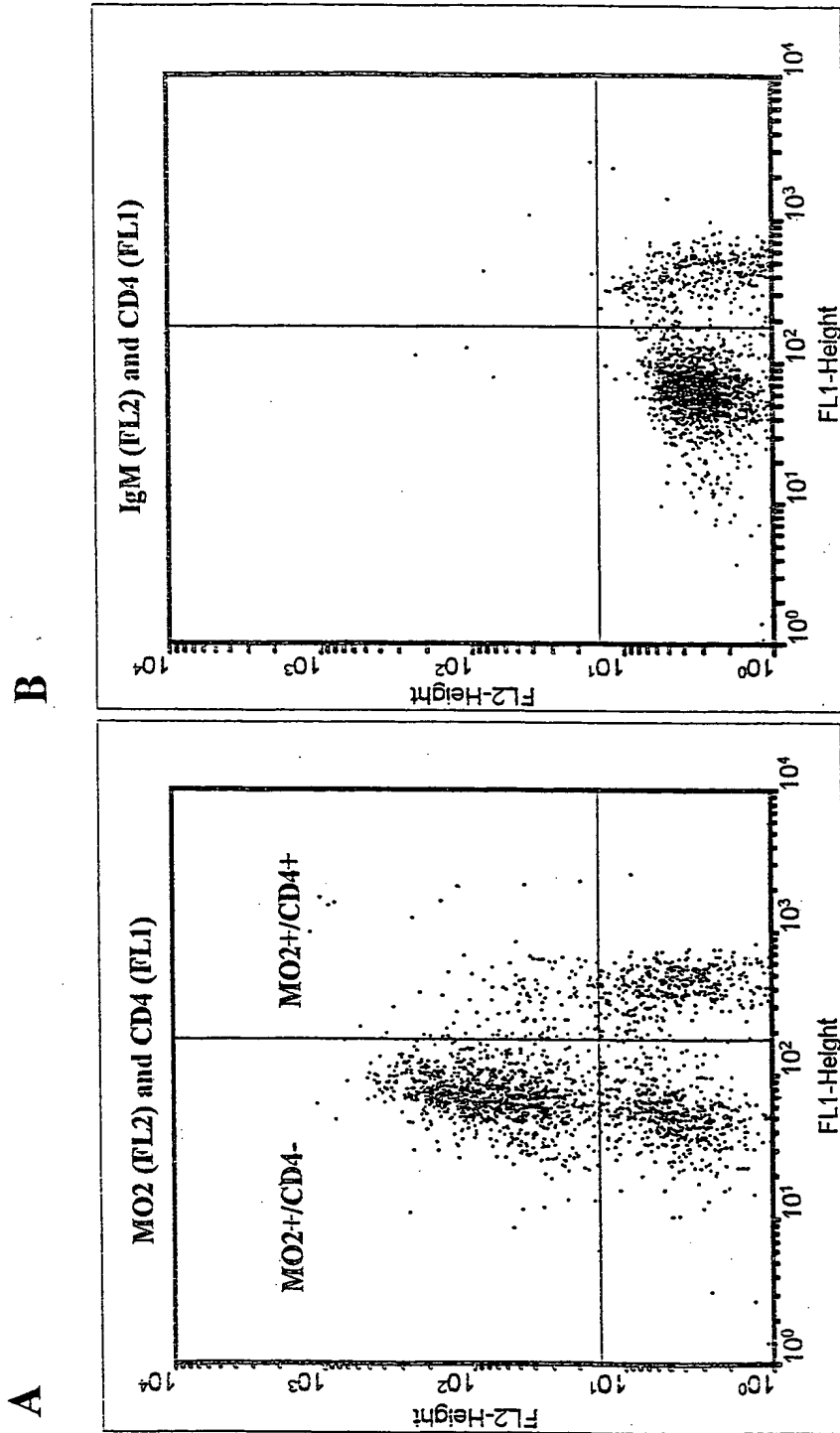


Fig. 6

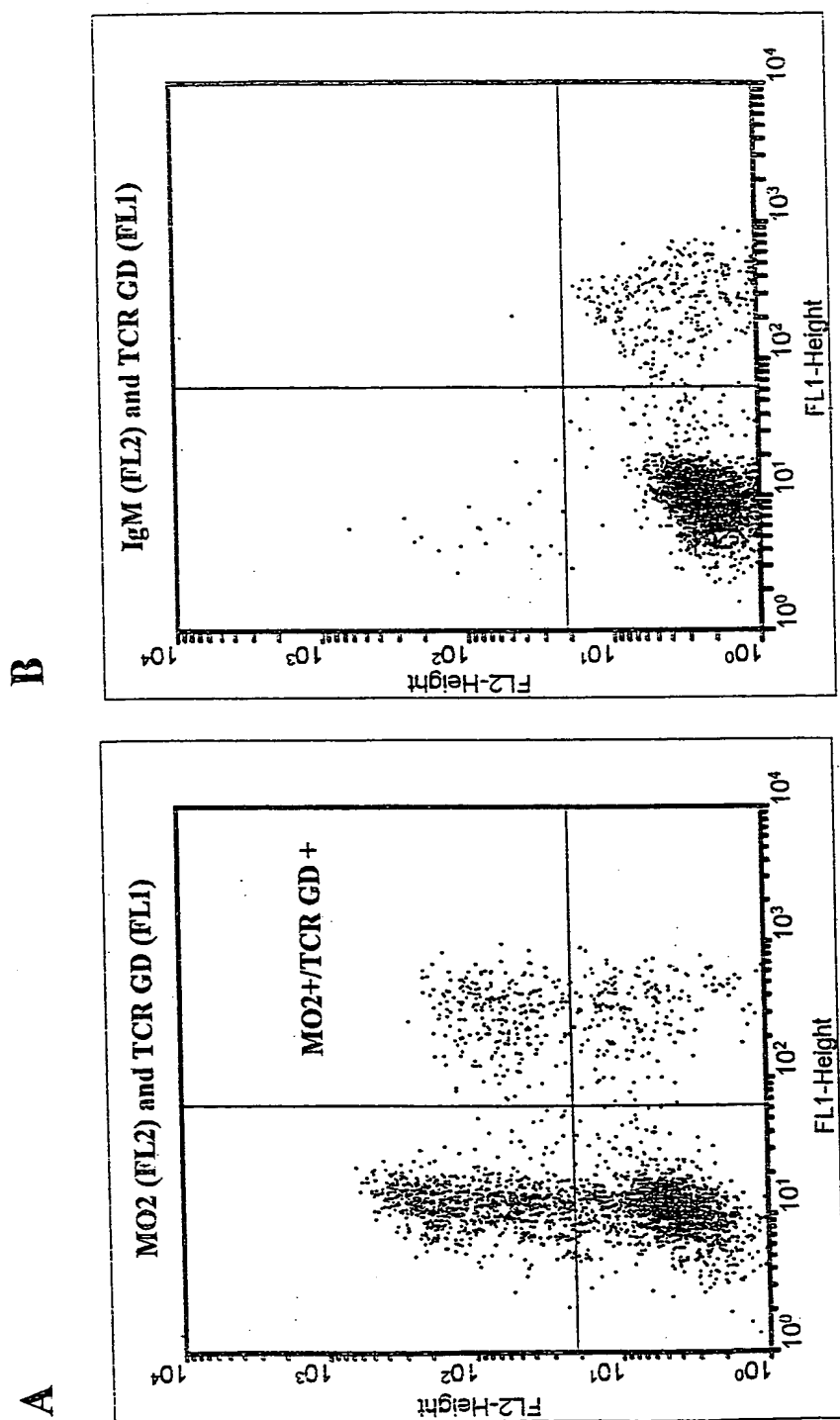


Fig. 7

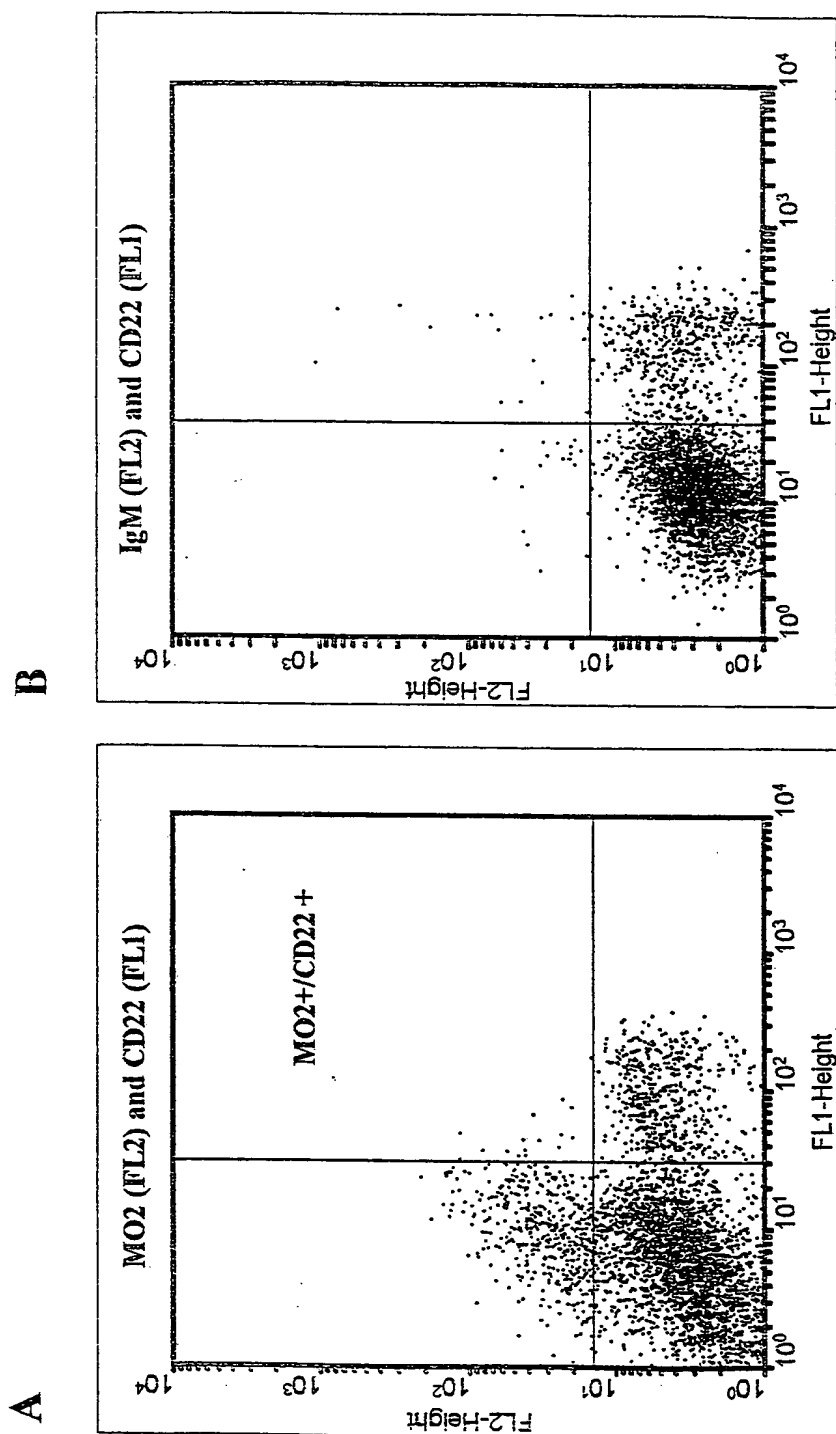


Fig. 8

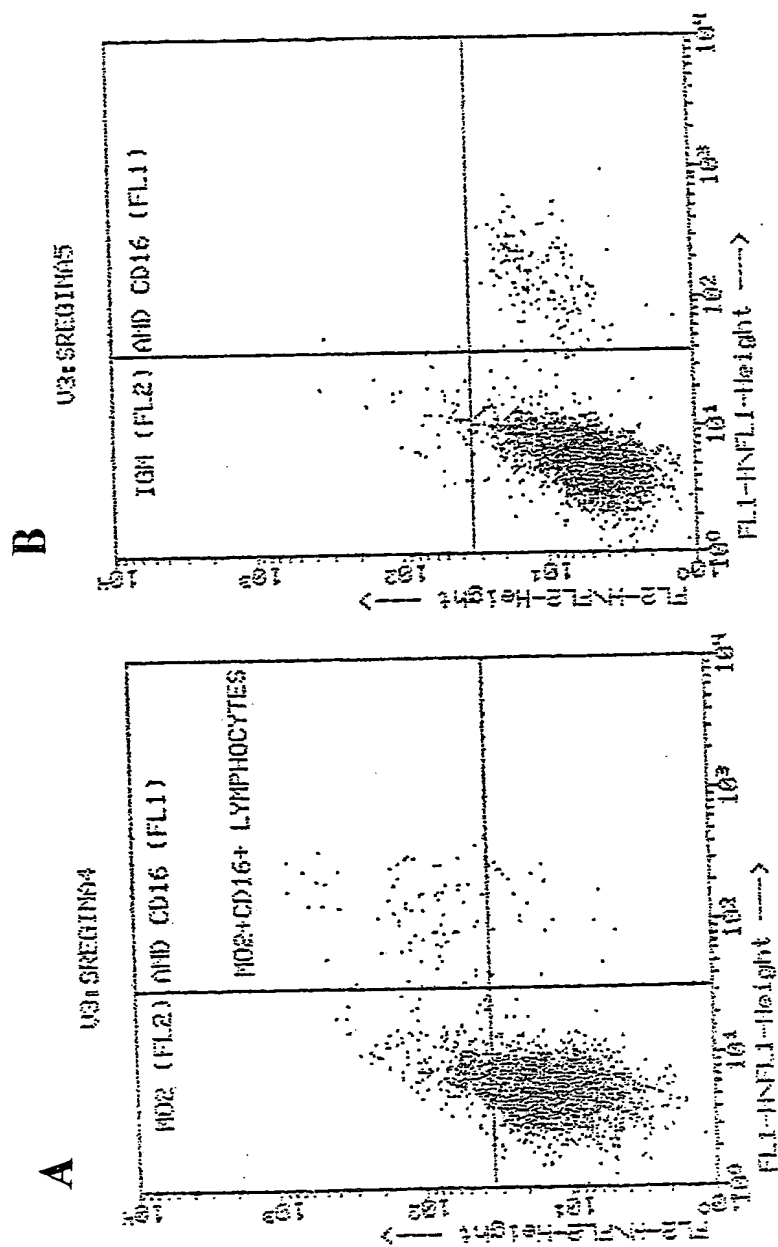
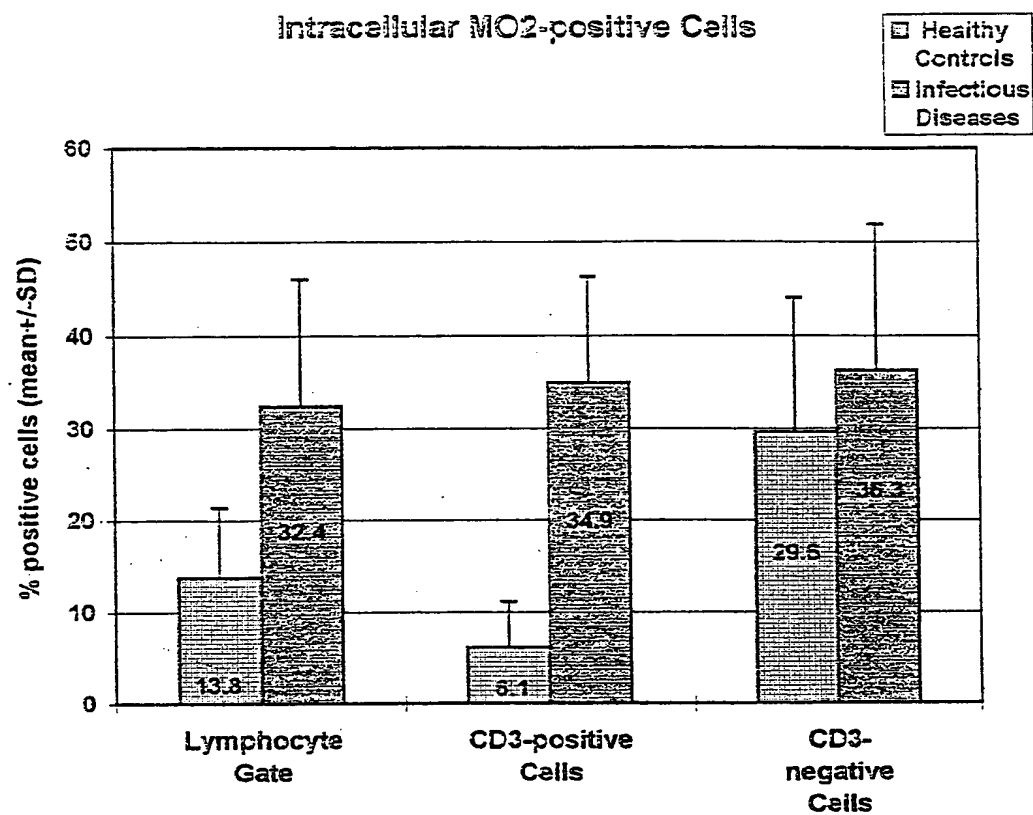


Fig. 9

**Fig. 10**

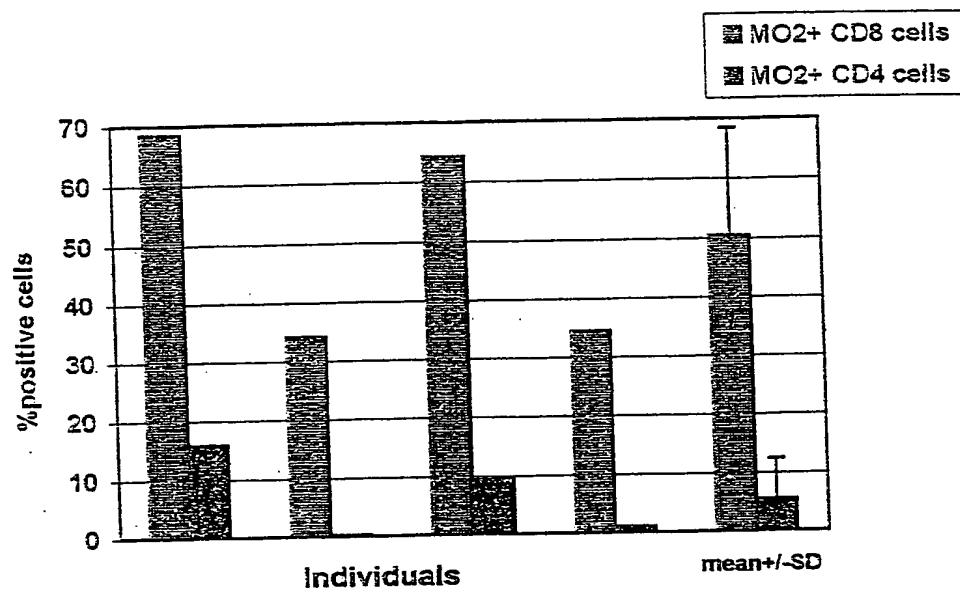


Fig. 11

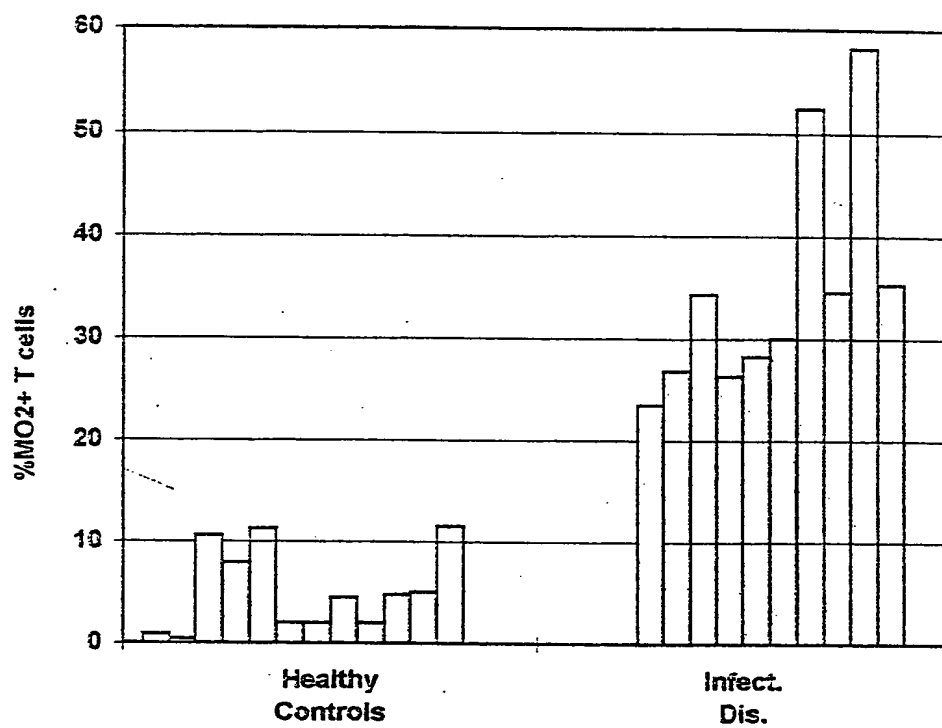


Fig. 12

